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13. ABSTRACT *(Maximum 200 words)*

Matrix metalloproteinases (MMPs) contribute to cancer progression. Here we show that MMP-3/stromelysin-1 (Str1) can promote both tumor initiation and epithelial-to-mesenchymal conversion seen in advanced cancers. Transgenic mice that expressed Str1 in mammary epithelium developed fibrosis (77%), hyperplasias (64%), dysplasias (20%) and carcinomas (7%), whereas non-transgenic controls developed only mild fibrosis and/or hyperplasias (<10%). When functionally normal cultured mammary epithelial cells were transfected with a tetracycline (Tet)-repressible Str1 expression vector, Str1 induction resulted in E-cadherin cleavage, scattered growth, replacement of cytokeratins by vimentin, and acquisition of the ability to invade Matrigel and grow in soft agar. These cells, when injected into surgically cleared mammary fat pads of immunodeficient mice, formed ductal structures when Str1 expression was repressed by adding Tet to the drinking water, but formed vimentin-positive, spindle-cell tumors in 30% of injected sites when Str1 was induced. Large tumors grew at all sites when the cells were preinduced before injection, even with Tet present. cDNA array profiling showed that Str1 caused coordinated changes in the expression of intermediate filament markers and regulators of cell cycle progression, apoptosis and cell-matrix interactions. Our data suggest that, by altering the cellular microenvironment, Str1 can regulate the expression of genes that control cancer development.

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Introduction

Epithelial cancers are invariably associated with the induced expression of extracellular matrix (ECM)-degrading matrix metalloproteinases (MMPs) by the genetically normal stromal cells that, together with the genetically altered neoplastic cells, make up the tumour mass[1]. Recent evidence indicates that MMPs are not only key agonists in tumour angiogenesis, invasion and metastasis[1], but may also alter susceptibility to tumour development[2-4]. But how do MMPs alter neoplastic risk? Here we report that expression of the MMP-3/stromelysin-1 *(Str1)* gene can promote premalignant changes and mammary tumourigenesis in transgenic mice, and can convert phenotypically normal mammary epithelial cells into highly infiltrative tumours *in vivo*. Our results indicate that disruption of the cellular microenvironment by *Str1* promotes the development of both early premalignant lesions and the later stages of neoplasia associated with more aggressive malignant behaviour. Thus targeting this stromal factor during the premalignant phases of tumour development may prevent malignant conversion.

Body

To test the hypothesis that stromal MMPs can have oncogenic effects, we chose *Str1* because it was originally cloned as a tumour-specific gene, and yet is highly regulated in normal mammary gland development[1,5-8]. Str1 can degrade numerous ECM substrates, activate other MMPs and inactivate several serine proteinase inhibitors[1]. It is expressed in stromal cells throughout mammary development and maximally expressed during involution when ECM remodeling and alveolar regression take place[5-8], and it induces apoptosis in mammary epithelial cells in culture[9]. Although stromal cells synthesize Str1, the protein is frequently associated with the epithelium[5]. Accordingly, transgenic mice expressing an autoactivating isoform of rat *Str1* targeted to mammary epithelium show increased ductal branching and precocious lobulo-alveolar development in virgin mice[7,10]. During pregnancy and lactation, the *Str1* transgene expression

induces upregulation of endogenous *Str1* in surrounding stromal fibroblasts, and leads to fibrosis, neovascularization and expression of tenascin-C, all of which are hallmarks of the reactive stroma of involution[8]. As a result, basement membrane integrity is lost and precocious involution occurs during pregnancy, resulting in collapsed alveoli and low milk protein production in lactation[10,11]. Crossing the *Str1* transgenic mice with mice overexpressing a tissue inhibitor of metalloproteinases-1 *(Timp1)* transgene driven by the constitutive b-actin promoter abolishes the ECM degradation and unscheduled apoptosis[11].

An altered stromal phenotype is also characteristic of tumour progression. When we examined *Str1* transgenic mice over 6 months of age we found not only an altered stroma, but also a number of other unexpected changes (Table 1). Of 163 mice from five independent transgenic lines, 77% exhibited moderate or severe fibrosis (accumulation of interstitial collagen and fibroblasts), 53% had lymphocytic inflammatory infiltrates, 64% had moderate or severe hyperplasias, 20% had atypical hyperplasias or ductal carcinoma *in situ*, and 7.4% developed mammary carcinomas. In addition, reactive mammary lymph nodes and lymphoproliferative disease/lymphomas that appeared to originate in mammary lymph nodes were seen in 27% of *Str1* transgenic mice. The incidence of these lesions was 1.2-1.9-fold higher in parous mice than in virgin mice (Table 1), and the hyperplastic and fibrotic changes were generally more severe in the parous subset of animals. The mammary carcinomas included nine well-to-moderately well differentiated adenocarcinomas (Fig. 1a-c), and three undifferentiated tumours that showed evidence of epithelial-to-mesenchymal transitions (EMT). One of these was metastatic and coexpressed both epithelial cytokeratins (CKs) and the mesenchymal marker vimentin (Vim) (Fig. 1d-f). A cell line (TCL-1) established from this tumour continued to express both intermediate filament types and formed highly invasive spindle-cell tumours that remained CK- and Vim-positive *in vivo* (Ref. 12 and unpublished data). The two remaining tumours were carcinomas with sarcomatous metaplasia (carcinosarcomas) that contained CK-positive and Vim-negative carcinomatous cell populations, and distinct Vim-positive and CK-negative sarcomatous populations (Fig. 1h-j). Because carcinosarcomas are rare in mice[13] and account for only about

0.1% of all human breast cancers[14], their rate of occurance in the *Str1* transgenic mice appeared unusually high.

In contrast to the transgenic mice, only a few of 94 nontransgenic control mice developed mild fibrosis, hyperplasia or lymphoid infiltrates and none of the other more severe lesions (Table 1). We also isolated a subline of *Str1* transgenic mice in which expression of the transgene was silenced and could not be detected by RT-PCR, presumably due to methylation of the transgene as determined by altered sensitivity to restriction endonucleases and its stable transmission to subsequent litters. Like the nontransgenic littermate controls, the nonexpressing transgenic mice exhibited a low incidence of mild hyperplasia, fibrosis and lymphocytic infiltrates (Table 1). The low incidence rates for these mild lesions were similar for virgin and parous controls.

Two distinct mechanisms could account for the resulting tumours: *Str1* could act directly on the epithelial cells to promote genomic instability, or altered stromal-epithelial interactions could favour selection of genetically altered epithelial cells that then undergo neoplastic progression. To test the first hypothesis we treated an immortal, but functionally normal mouse mammary epithelial cell line (Scp2) with recombinant Str1. The Str1 rapidly induced EMT in the epithelial cells, characterized by loss of CKs and upregulation of Vim (Fig. 2). We then stably transfected Scp2 cells with the autoactivating rat *Str1* cDNA described above, but under the control of a tetracycline (Tet)-repressible promoter[15]. When grown in the presence of reconstituted basement membrane (Matrigel), lactogenic hormones and Tet, the transfected *Str1*-expressing clones p2S7 and p2S10 were just as capable of forming polarized alveolar structures and producing milk proteins as the parental Scp2 cells and the nonexpressing p2S3 clone. Induction of *Str1* expression by removal of Tet resulted in downregulation of CKs, upregulation of Vim (Fig. 2), acquisition of a scattered morphology, cleavage and loss of E-cadherin, loss of the ability to undergo lactogenic differentiation, and acquisition of the ability to invade Matrigel and form anchorage-independent colonies in soft agar[15,16].

When parental Scp2 cells and *Str1* transfected clones were injected into surgically cleared (gland-free) mammary fat pads of SCID mice in the presence of Tet, they formed glandular duct-

like structures (Fig. 3a-d). However, if *Str1* expression was induced *in vivo* by removing Tet from the drinking water, p2S10 cells formed small spindle-cell tumours in about one-third of injected sites by 6 weeks (Table 2). When *Str1* expression was induced in culture for 2 months prior to injection, large tumours grew at all injected sites regardless of the presence or absence of Tet in the drinking water. The tumours were highly infiltrative, and although haematogenous metastases were not detected, one animal had multiple intraperitoneal tumours following invasion through the abdominal wall. Moreover, preinduced p2S10 cells were able to form ectopic tumours within more stringent subcutaneous sites (data not shown). Cells that were induced to express *Str1* for 6 days in culture continue to undergo progressive phenotypic conversion even when grown in the presence of Tet and the synthetic MMP inhibitor GM6001[15]. Likewise, when Tet was withheld from the drinking water for 12 days after injecting uninduced p2S10 cells, and then replaced for the remaining 4.3 weeks, one tumor formed out of eight orthotopic sites, suggesting that once tumorigenicity was achieved, it could no longer be blocked by repressing *Str1* expression. Unlike the CK-positive and Vim-negative pseudoglandular growths that formed when *Str1* expression was repressed, all tumours were composed of Vim-positive and CK-negative spindle-shaped (mesenchymal-like) cells, with fewer than 1% CK-positive cells remaining (Fig. 3e-h). Furthermore, not only had the tumours undergone EMT, but the mesenchymal-like cells in several tumours showed small areas of differentiation to a cartilage-like phenotype (chondroid metaplasia; Fig. 3f). Thus induction of *Str1* expression had rendered the transfected cells tumourigenic and could trigger EMT and invasive behaviour *in vivo* as well as *in vitro*.

Altered cell adhesion, such as that effected by *Str1*, may also promote genomic instability[17,18]. If the *Str1*-induced signals favour accumulation of genomically unstable cells, then the derived tumours could very well have nonrandom mutations. Indeed, comparative genomic hybridization (CGH) analysis of ten mammary lesions from three *Str1* transgenic lines revealed nonrandom DNA losses in specific regions of chromosomes 4 and 7 in several early and late lesions (Fig. 4A). In addition, a severe hyperplasia, the three high-grade tumours, and the TCL-1 cell line had DNA gains in chromosomes 6 and 15. Because these gains were associated with

EMT, we microdissected one carcinosarcoma to separately analyze its distinct populations. The chromosome 15 amplification was only present in microdissected sarcomatous areas, whereas other CGH changes were seen throughout the tumour. CGH profiles for non-tumoral tissues from the same mice and from histologically normal mammary glands from two transgenic mouse lines were invariably normal. Chromosome 15 gains were also seen . . . (Fig. 4B). Pending CGH data. Identical gains in the mid-distal portion of chromosome 15 were seen in both microdissected chondroid and spindle-cell areas, but not in adjacent normal stroma, indicating that both areas arose from injected rather than host cells. These data suggest that the altered loci may contain recessive- and dominant-acting genes that contribute to early and late cancer progression, respectively. They also support the hypothesis that MMPs can produce an abnormal stromal environment within which clones of epithelial cells containing selected mutations may accumulate.

Stromal MMPs can effect cellular signaling by several routes[17,19]. They alter cell-matrix interactions and release bioactive ECM fragments[17]; they can cleave a growing list of cell-surface proteins, including E-cadherin, a known contributor to cancer development[18]; they release growth factors, angiogenic factors and their inhibitors from the ECM and cell surface[20]; they can cause recruitment of other host cells[8]; and they may promote genomic instability by altering cell cycle checkpoint controls[18]. Thus MMPs may impact all stages of cancer progression. Recent evidence indicates that some inherited cancer syndromes result from "landscaper" defects that first affect stromal cells rather than adjacent epithelia[21]. Accordingly, MMPs elaborated during stromal remodeling and inflammation may promote neoplastic transformation of otherwise normal cells, or they may promote the effects of carcinogens and pre-existing gene defects. For example, wild-type fibroblasts foster the tumourigenicity of human MCF7 breast cancer cells in nude mice, yet fibroblasts lacking MMP-11/stromelysin-3 do not[2]. Moreover, mice carrying the *Apc*^{Min} mutation develop fewer and smaller intestinal adenomas if rendered deficient in MMP-7/matrilysin[3], and transgenic expression of MMP-1/collagenase-1 in mouse skin leads to hyperproliferative lesions and a greater sensitivity to chemical carcinogens[4], while MMP-11 null mice have a reduced

sensitivity to carcinogens[2]. By extension, the tumour promoter activity of phorbol esters may, in part, stem from their ability to upregulate stromal MMP expression.

Our data indicate that *Str1* can trigger EMT, a process seen in high grade cancers[22,23] and during embryonic development and wound repair when otherwise adherent epithelia become migratory and invasive[24,25]. Indeed, the most aggressive human breast cancer cell lines lack E-cadherin and coexpress CKs and Vim[26], and Vim is preferentially expressed in those breast cancers with poor prognostic indicators[27,28]. Furthermore, when tumour cells undergo EMT, they then synthesize MMPs that are otherwise confined to stromal cells[12,29,30]. Thus our data indicate that *Str1* expression can promote early neoplastic changes, stereotyped genomic changes, and late phenotypic conversions associated with more aggressive tumour behaviour. We favour the emerging concept that an altered stromal environment can predispose toward neoplastic transformation. Because *Str1* can promote both early and late cancer progression, inhibition of this enzyme during any stage of tumour evolution may slow or halt further disease progression.

Methods

Transgenic Mice. CD-1 mice with an autoactivating rat *Str1* transgene targeted to mammary epithelium by the murine whey acidic protein gene promoter were generated as described[8].

Tumourigenicity Assay. Cell culture and immunocytochemistry were performed as described[15]. The developing parenchyma of abdominal (#4) mammary glands was removed from weanling SCID mice[31] and 1 x 10^6 Scp2 or p2S cells in serum-free medium were injected into residual gland-free mammary fat pads or subcutaneously at the nape of the neck. Mice were maintained for 6 or more weeks with or without 10 mg/ml Tet in their drinking water. Tumour volumes were calculated as (length x width^2)/2.

Histopathology. Mammary wholemounts[8] were photodocumented and reprocessed for paraffin embedment. Alcian blue staining was by the method of Hall[32]. Antigen retrieval was by brief 0.4 mg/ml proteinase K digestion for Vim, or by microwave heating in citrate buffer (BioGenex). Before adding peroxidase (HRP)-conjugated reagents, endogenous peroxidase activity was

blocked with a methanol/H_2O_2 solution. Immunolocalization was by rat anti-mouse CK-8 (a gift from Dr. Rolf Kemler; 1:50) and biotinylated rabbit anti-rat IgG (Vector Laboratories; 1:200), HRP-conjugated mouse anti-cow Vim (DAKO; prediluted), or biotinylated rat anti-mouse smooth muscle actin (a gift from Dr. Leif R. Lund; 1:50). Biotinylated antibodies were detected with avidin-biotin-HRP complexes. HRP activity was visualized with diaminobenzidine (DAB) and nuclei were counterstained with Meyer's haematoxylin.

Comparative Genomic Hybridization. DNAs were extracted from cultured cells, frozen tissues, or paraffin blocks by standard methods, or from lightly stained paraffin sections after laser capture microdissection[33]. Reference and test DNAs labeled with Texas red-5-dCTP and fluorescein-12-dCTP, respectively, were hybridized to normal metaphase chromosome spreads, chromosomes were identified by 4,6-diamino-2-phenylindole (DAPI) counterstaining, and green:red fluorescence intensity profiles were obtained as previously described[34].

Additional, failed or unfinished experiments.

Effect of GM6001 on Tumourigenicity. To test the effect of an MMP inhibitor on tumourigenicity, mice injected with p2S10 cells were maintained without Tet in their drinking water and instead given daily intraperitoneal doses of the MMP inhibitor GM6001 or its inactive analog GM1210 (100 mg/kg). This study was discontinued because the inhibitor apparently interfered with wound healing after mammary fat pad clearance, and resulted in untoward morbidity and mortality not seen in the GM1210 control animals.

Effect of Str1 on other cell lines. Other mouse mammary epithelial cell lines (NMuMG, Comma1D and EPH-4) were treated with recombinant human Str1 (a gift of Dr. M. Navre) for six days in defined medium. Immunocytochemistry revealed that Str1 had caused loss of desmosomal desmoplakins in over 50% of NMuMG cells, but had no effect on CK, Vim, E-cadherin, β-catenin, occludin or ZO-1 expression patterns in these cells. Although EPH-4 cells were relatively difficult to remove by trypsinization, they were readily removed from tissue culture plastic by Str1, suggesting the cleavage of an important adhesion molecule in these cells by Str1. Still, low-dose

treatment of EPH-4 cells had no effect on CK, Vim, E-cadherin, occludin, or desmoplakin expression in these cells. Whereas Comma1D cells were either immunoreactive for CKs alone, or coexpressed CKs and Vim, Str1-treated cells either coexpressed both intermediate filament types, or expressed only Vim, again suggesting EMT.

Effect of other MMPs on Scp2 cells. Unlike Str1, purified gelatinases A and B (MMPs 2 and 9) had no appreciable effect on CK, Vim, E-cadherin, β-catenin, occludin, ZO-1, cortactin or zyxin immunoreactivity after six days in culture, but resulted in lost or weakened staining for desmoplakins 1 and 2.

Effect of Str1 on E-cadherin/β-catenin signaling. Because Str1 can cause the cleavage of E-cadherin which, in turn, interacts with β-catenin, because β-catenin interacts with the APC and Wnt-1 signaling pathways, because β-catenin can be shuttled to the nucleus by Lef/Tcf-1, and because β-catenin/Lef complexes can effect gene transcription, we examined the possible role of Str1-induced E-cadherin cleavage in transcriptional regulation. Initial transient transfection experiments using a *fos* promoter-driven luciferase reporter construct containing upstream Lef/Tcf-1 recognition sequences (a gift of Dr. R. Grosschedel), failed to show any differences in luciferase activity in p2S10 cells grown with or without Tet. These results were most likely due to the high constitutive expression of the *fos* promoter in these cells, thus these experiments are presently being performed using a fly alcohol dehydrogenase promoter-driven CAT reporter construct containing upstream wild-type or mutant Lef recognition sequences.

Effect of Str1 on gene expression. To identify genes that are induced or repressed during Str1-driven EMT, RNAs were isolated from p2S10 cells grown with Tet ot without Tet for 6 days or 3 months. Differential expression was then examined using mouse cDNA arrays or differential display, and confirmed by RT-PCR. For cDNA array profiling, polyA+ RNA was obtained and ^{32}P-labeled cDNA probes were made using reverse transcriptase. These were then hybridized to membranes containing an array of duplicate, immobilized cDNAs of interest, and computer-based comparisons of their signals were obtained. As had been seen at the protein level, Vim gene expression was progressively upregulated by *Str1* induction, while CK-18 expression was

progressively downregulated. Genes for the cell cycle regulator cyclin-D1 and the FAS and apoptosis regulator TDAG51 were upregulated early (at 6 days) and remained upregulated at 3 months. Other genes showed either transient upregulation (eg., the gene for the lysosomal enzyme and poor prognostic indicator cathepsin D), early stable repression (eg., the gene for the putative tumour suppressor Egr-1), early transient repression (eg., the gene for the transcription factor TTF1), late repression (eg., the gene for the attenuator of ErbB2 Tob), or no change in expression (eg., the gene for the putative metastasis suppressor nm-23).

Conclusions

Several MMPs play a major role in late cancer progression, and new data suggest that some may contribute to early tumor development as well. However, the mechanisms whereby MMPs can regulate tumorigenesis are incompletely understood. Here we have shown that the MMP Str1 can trigger early events involved in tumor initiation and can induce epithelial-to-mesenchymal transformations that are often seen in more advanced aggressive cancers. Gene expression profiling using cDNA arrays has also shown that Str1 can trigger coordinated changes in the expression of several genes, including intermediate filament markers and regulators of cell cycle progression, apoptosis and cell-matrix interactions. These data suggest that Str1 and perhaps other MMPs may promote tumorigenesis at both early and late stages by altering cell-cell and cell-matrix interactions and by modulating the expression of genes that control tumor development.

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Note: This report contains unpubl;ished data.

Table 1 Incidence of mammary gland pathologies in *Str1* transgenic mice.

	Nontransgenic Controls	Nonexpressing Transgenics	Virgin Transgenics	Parous Transgenics	All Expressing Trangenics
N	94	75	105	58	163
Median Age (mos.)	14	13	18	17	18
Age Range (mos.)	6-24	6-23	7-28	6-28	6-28
No Pathology	85 (90%)	62 (83%)	17 (16%)	3 (5.2%)	20 (12%)
Fibrosis	7 (7.4%)	8 (11%)	74 (70%)	51 (88%)	125 (77%)
Hyperplasia	4 (4.3%)	3 (4%)	65 (62%)	40 (69%)	105 (64%)
Atypical Hyperplasia	0 (0%)	0 (0%)	18 (17%)	15 (26%)	33 (20%)
Carcinoma	0 (0%)	0 (0%)	6 (5.7%)	6 (10%)	12 (7.4%)
Lymphoid Infiltrates	3 (3.2%)	7 (9.3%)	51 (49%)	36 (62%)	87 (53%)
Lymphoid Abnormalities*	0 (0%)	0 (0%)	22 (21%)	22 (38%)	44 (27%)

* Lymph node abnormalities included sinus histiocytosis, medullary plasmacytosis and lymphomas.

Table 2 Tumorigenicity at 6 weeks after orthotopic injection.

	+ Tetracycline		-Tetracycline	
Cell Line	Incidence (tumors/site)	Volume (cm^3) (mean ± SEM)	Incidence (tumors/site)	Volume (cm^3) (mean ± SEM)
Scp2	ND*	-	0/10	-
p2S3	0/6	-	0/6	-
p2S7	0/6	-	0/6	-
p2S7-Pre†	6/6	0.25 ± 0.09	6/6	0.22 ± 0.08
p2S10	0/14‡	-	5/18‡	0.04 ± 0.01§
p2S10-Pre†	8/8	0.69 ± 0.13	8/8	1.05 ± 0.18§

* ND, not determined

† Pre, preinduced for 2 mos. prior to injection

‡ P=0.04 (1-tailed Fisher exact test)

§ P=0.0006 (t-test)

Note: This report contains unpublished data.

Figure Legends

Figure 1 Histopathologic and immunohistochemical appearance of mammary gland tumours from *Str1* transgenic mice. **a-c,** Moderately well differentiated adenocarcinoma. **d-f,** renal metastasis from an undifferentiated carcinoma. **g-i,** Carcinosarcoma. A, adjacent stroma; C, carcinomatous area; K, normal kidney; S, sarcomatous area. H&E, haematoxylin-eosin stains. Scale bar, 100 mm.

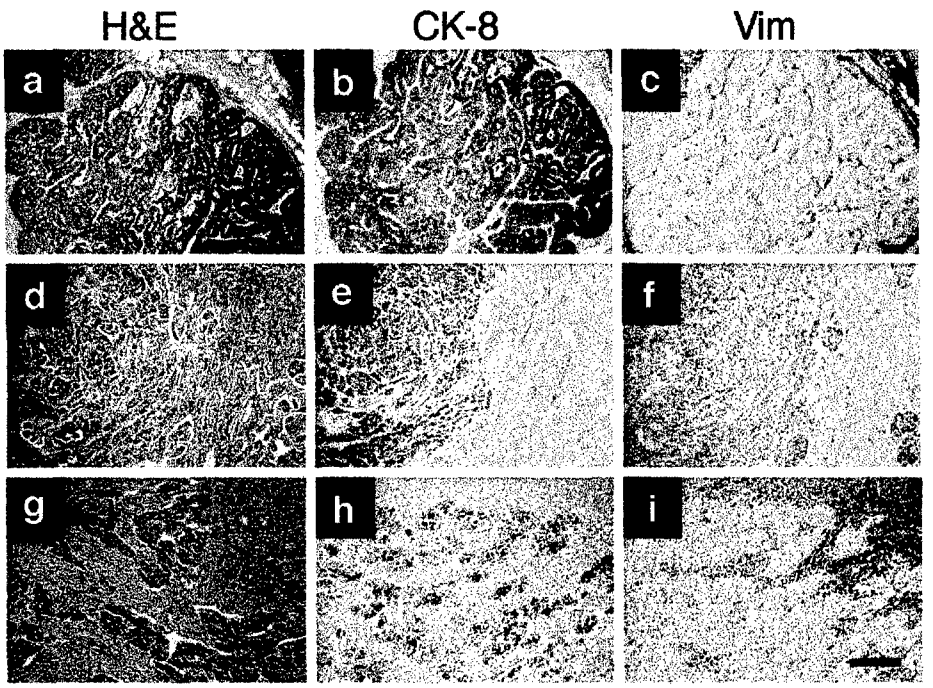
Figure 2 Effect of Str1 on intermediate filament expression. Scp2 cells were maintained for 6 days in the absence (**a**) or presence (**b**) of trypsin-activated recombinant Str1 and stained by indirect immunofluorescence[16] for CKs (red) and Vim (green). Nuclei were counterstained with DAPI (blue). Scale bar, 50 mm.

Figure 3 Histologic appearance of *Str1*-transfected p2S10 cells grown in cleared mammary fat pads. **a-d,** Appearance of gland-like cysts and duct-like structures that form when *Str1* expression is repressed by Tet *in vivo*. **e-h,** Appearance of spindle-cell tumours that form when *Str1* expression is induced *in vivo* or before transplantation by Tet withdrawal. C, alcian blue-positive chondroid area; V, vascular smooth muscle cells. Scale bar, 200 mm (**a**), 50 mm (**b-d**) or 100 mm (**e-h**).

Figure 4 CGH profiles. **A.** Genomic changes in individual mammary gland lesions from *Str1* transgenic mice. Approximate locations of DNA gains (green) and losses (red) are indicated along otherwise unaltered (yellow) chromosomes, with black circles representing acrocentric centromeres. All adjacent stromal and non-mammary control tissues had normal CGH profiles. Hyp, hyperplasia; AH, atypical hyperplasia; Ca, carcinoma; Met, metastatic; CaSa, carcinosarcoma; *, gain seen in sarcomatous areas only. **B.** Normalized fluorescence intensity profiles for chromosome 15 obtained with DNA isolated from uninduced p2S10 cells (**a**)(data pending), microdissected spindle-cell areas from a p2S10-derived tumour (**b**), chondroid areas from the same tumour (**c**), and normal stroma adjacent to the same tumour (**d**). Average green:red fluorescence ratios (heavy lines) ± 1 standard deviation (thin lines) are shown for the number of metaphase spreads examined (n). The dashed horizontal lines, and upper and lower dotted lines indicate fluorescence ratios of 1, 1.5, and 0.5, respectively.

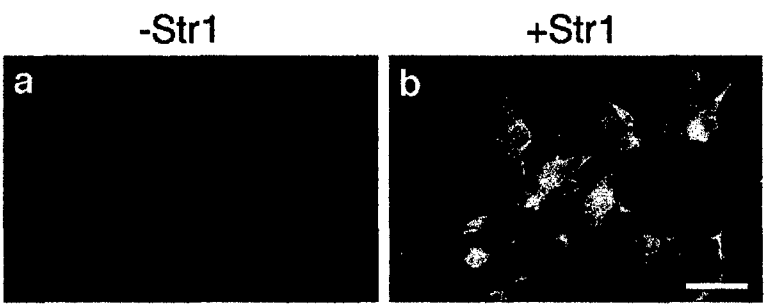
Note: This report contains unpublished data.

Figure 1



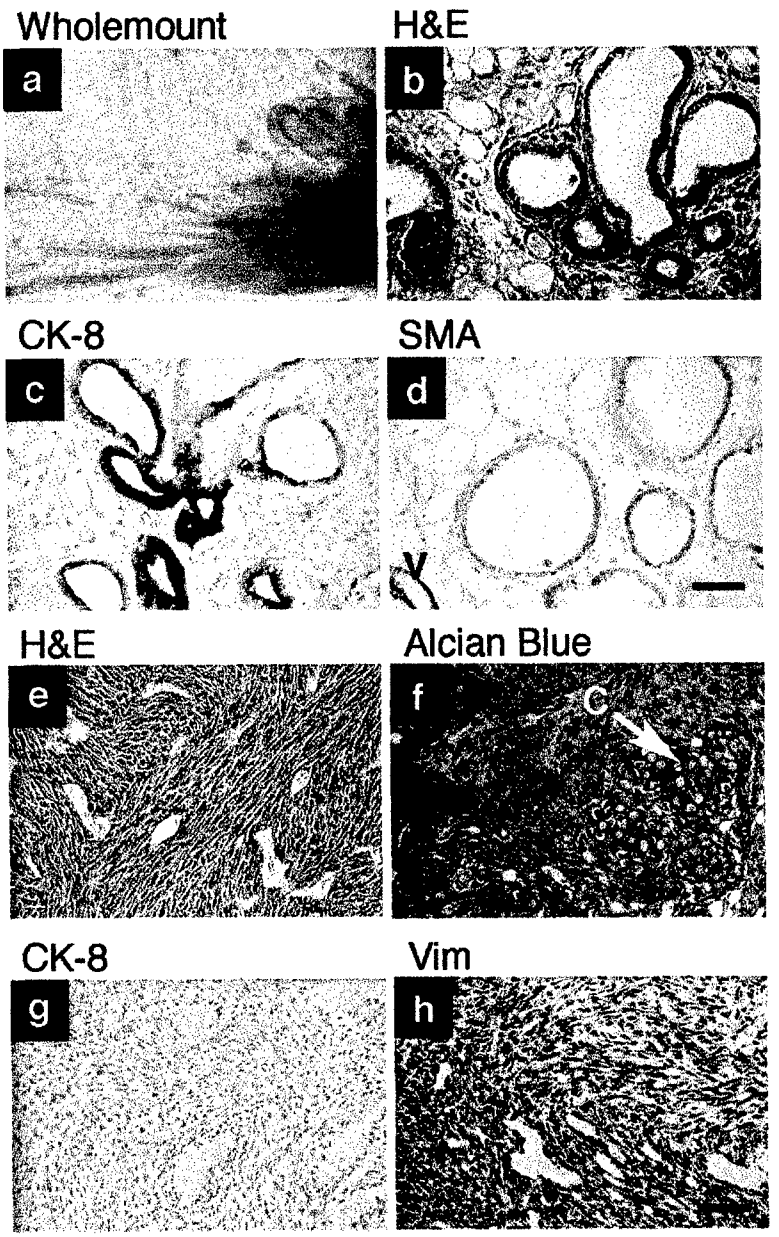
Note: This report contains unpublished data.

Figure 2



Note: This report contains unpublished data.

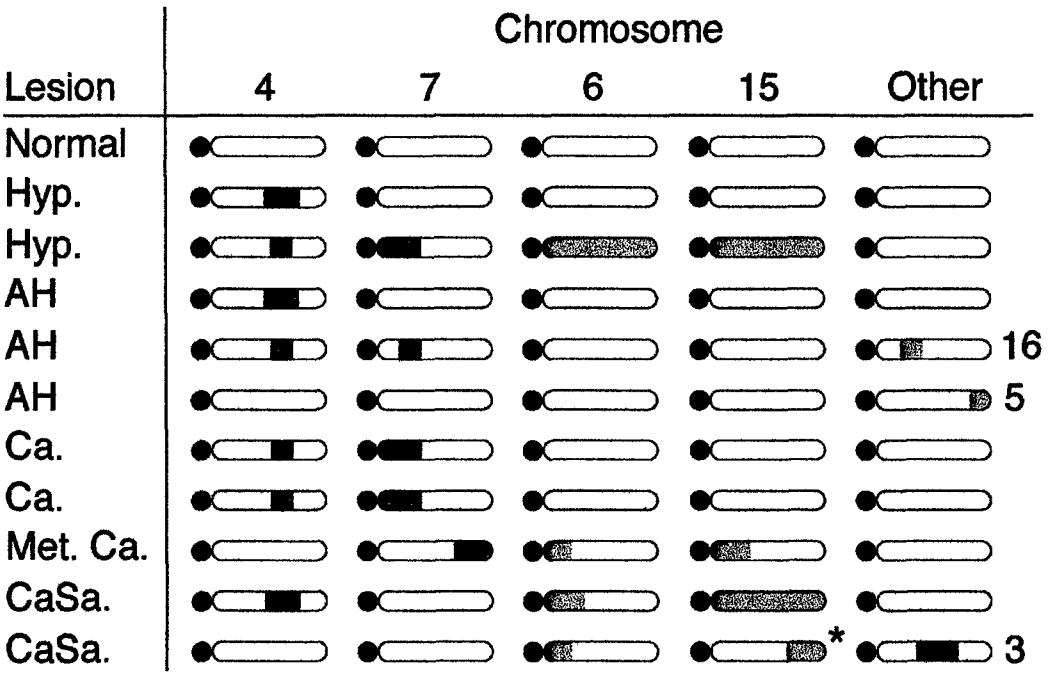
Figure 3



Note: This report contains unpublished data.

Figure 4

A



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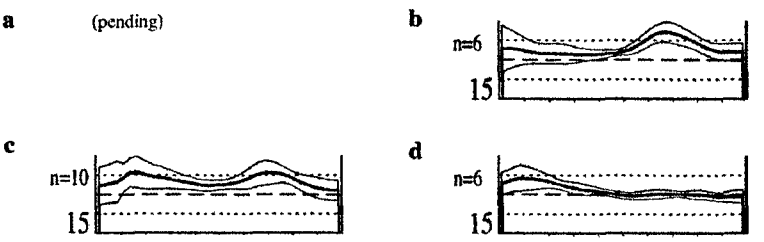

DEPARTMENT OF THE ARMY
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SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports. Request the limited distribution statement for reports on the enclosed list be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

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FOR THE COMMANDER:

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management

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